Cold cases in epidermolysis bullosa: not the usual suspects

Turcan, Iana

Epidermolysis bullosa simplex with mottled pigmentation associated with *EXPH5* mutations

Iana Turcan[1], MD; Anna M. G. Pasmooij[1], PhD; Peter C. van den Akker[1,2], MD, PhD; Henny Lemmink[2], PhD; Richard J. Sinke[2], PhD, professor; Marcel F. Jonkman[1], MD, PhD, professor

[1]Centre for Blistering Diseases, Department of Dermatology, University of Groningen, University Medical Centre Groningen, Groningen, The Netherlands
[2] University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, the Netherlands

Published in JAMA Dermatology 2016 Oct 1;152(10):1137-1141

List of abbreviations:

DDD: Dowling Degos disease

DPR: dermatopathia pigmentosa reticularis

EB: epidermolysis bullosa

EBS: epidermolysis bullosa simplex

EBS-AR: epidermolysis bullosa simplex autosomal recessive

EBS-MP: epidermolysis bullosa simplex with mottled pigmentation

GS: Griscelli syndrome

NFJS: Naegelli-Franceschetti-Jadasson-syndrome

Importance

Epidermolysis bullosa simplex (EBS) is a group of clinically and genetically diverse mechanobullous genodermatoses characterized by the fragility of skin and mucous membranes. Recently, mutations in *EXPH5* encoding exophilin-5 (also known as Slac2-b, an effector protein involved in intracellular vesicle trafficking and exosome secretion) have been implicated in the pathophysiology of EBS. Here we report a novel homozygous nonsense mutation in *EXPH5* responsible for an EBS subtype with mottled pigmentation.

Main Outcome(s) and Measure(s)

Clinical examination and investigation of the molecular basis of patient's skin fragility and mottled pigmentation phenotype. Electron microscopy studies described the underlying pathology on an ultrastructural level.

Results

The clinical phenotype is characterized by mild generalized skin fragility, trauma-induced skin blistering since infancy, and development of remarkable diffuse mottled pigmentation on the trunk and proximal extremities. Sequencing the complete set of genes associated with epidermolysis bullosa revealed a homozygous nonsense mutation in exon 6 of *EXPH5*: c.3917C>G, p. Ser1306*. Electron microscopy revealed disruption of keratin filament cytoskeleton and accumulation of melanosomes in a disordered distribution in the keratinocytes.

Conclusions and relevance

The current study illustrates the first clinically well-documented, mottled pigmentation phenotype related to a novel *EXPH5* mutation. In addition, by means of electron microscopy image analysis, it proposes a hypothesis for the pigmentary changes in this rare autosomal recessive EBS subtype. These findings expand the genetic and phenotypic spectrum of human inherited skin fragility disorders and proposes the addition of EBS resulting from *EXPH5* mutations to the EBS-mottled pigmentation subtype.

Introduction

Basal epidermolysis bullosa simplex (EBS) represents a group of inherited skin fragility disorders that result from mutations in *KRT5, KRT14, PLEC, DST, COL17A1, ITGB4,* and *EXPH5* genes.[1] To date, only four pedigrees with EBS resulting from *EXPH5* (MIM 612878) mutations have been described (Figure 1A).[2-5] *EXPH5* encodes exophilin-5, also called synaptotagmin-like protein lacking c2 domains b (Slac2-b), an effector protein of the Rab27B GTPase. Slac2-b plays a role in intracellular vesicle trafficking and exosome secretion.[2,6] The clinical phenotype of the affected individuals manifested as inherited skin fragility that improved with age and mainly acral blistering with hemorrhagic crusts. [3-5]

Herein we describe a patient with EBS and a mottled pigmentation (MP) phenotype resulting from a novel homozygous nonsense mutation in *EXPH5*. In addition, we propose a hypothesis on the etiology of the pigmentary disturbances in this rare autosomal recessive EBS subtype.

Report of a Case

The index patient visited our clinic in her mid-teenage years. She was of Moroccan origin, born from a consanguineous union (Figure 1B). Fragility of the skin and predominantly acral blistering upon mechanical trauma were first noted when she was 1 year old. At 10 years of age she started developing remarkable diffuse MP on her trunk, axillae, and proximal extremities that was not related to skin blistering (Figure 2A, B). Skin fragility ameliorated with age; she developed trauma-induced blisters on her extremities only sporadically (Figure 2C). No other family members were affected.

Immunofluorescence microscopy of lesional skin with monoclonal antibody LL001 to keratin 14 revealed a cleavage plane through the basal keratinocytes, thus suggesting the diagnosis of basal EBS (Figure 1D). Staining with antibodies against integrin α6β4, BPAG-1e, plectin, type XVII collagen, laminin-332, and type VII collagen showed normal expression (not shown).

Electron microscopy revealed intercellular widening with loss of desmosomes in the basal and to some extent suprabasal layer (Figure 3A). In contrast, focally, large accumulations of desmosomes and striking cell membrane pliability were identified (Figure 3C). The basal keratinocytes showed consistent abnormalities in keratin cytoarchitecture with lateral aggregation and clumping of keratin filaments (Figure 3D). Few basal keratinocytes exhibited a ‘clear’ aspect with an obvious deficiency in the keratin cytoskeleton (Figure 3B). Some suprabasal cells showed retraction of keratin

filaments from the nuclear envelope (Figure 3A). Scattered within the keratinocytes was a large number of melanosomes and mitochondria of variable sizes (Figure 3D). The number of melanocytes was normal. Lamina densa showed some focal duplications and blind 'off-shoots', which represent remnants of the basement membrane after its reparation. Hemidesmosomes had normal appearance.

To identify the underlying genetic mutation, we applied our diagnostic next generation sequencing gene panel test consisting of a comprehensive set of 33 genes associated with or mimicking EB. The test is based on targeted SureSelect enrichment (Agilent Technologies Inc) and subsequent sequencing on a MiSeq sequencer (Illumina Inc). A novel homozygous mutation c.3917C>G, p. Ser1306* (GenBank NM_015065.2) in the last exon 6 of the *EXPH5* gene was identified and later confirmed by Sanger sequencing (Figure 1C). This mutation was not found in the Genome of the Netherlands[7], 1000 genomes, or the ExAc Browser databases.

Discussion

EBS-AR resulting from *EXPH5* mutations represents a very rare entity. The index patient is among the oldest individuals with *EXPH5* mutations reported up to now, which possibly allowed for the characterization of the MP phenotype, a late onset feature. The only previous mention of pigmentary changes in EBS associated with *EXPH5* mutations was in the original article of McGrath *et al.* which described subtle diffuse pigmentary skin mottling, similar (but less marked) to the pigment changes seen in Griscelli syndrome (GS).[2] The absence of photo documentation of the pigmentary changes does not allow for comparison with the MP phenotype in our patient. Given that in GS there is a pigmentary dilution in skin and hair[8], we consider the pigmentary changes described in the present study to be phenotypically different from those in GS. They resemble those seen in EBS-MP resulting from *KRT5* (c.74C>T, p.Pro25Leu; c.1649delG, p.Gly550Alafs*77) and *KRT14* (c. 356T>C, p.Met119Thr; c.1117_1158dup42, p.Ile373Glu386dup) mutations.[9] The possibility of EBS-MP was excluded through our extended EB gene panel test; no mutations in *KRT5* and *KRT14* were found. The homozygous mutation c.3917C>G; p. Ser1306* in *EXPH5* is located within exon 6 (Figure 1A, C). Located within the last exon, we expect this mutation not to activate the nonsense-mediated mRNA decay mechanism, and thus lead to the truncation of Slac2-b polypeptide. Nevertheless, considering the large size of exon 6, the mutation may also lead to protein instability and degradation.

Slac2-b has only recently been implicated in blistering disease and still little is known about its cellular functions. Latest studies indicate that Slac2-b is expressed in keratinocytes and other cell types involved in surface protection with an important role in microtubule vesicle transport and exosome secretion.[6] In regard to the mechanism of pigmentary changes in Slac2-b mutations it is appealing to investigate whether there is a relationship with the myosin-Va/ Rab27A/ Slac2-a family. Mutations in genes encoding these proteins are known to be involved in GS1, GS2 and GS3, respectively[8]. The pigmentary defect in GS arises from the failure to transfer mature melanosomes from melanocytes to the neighboring keratinocytes. Surprisingly, ultrastructural examination of our patient's skin revealed an accumulation of mature melanosomes in the keratinocytes (Figure 3D). They exhibited a scattered distribution throughout the cell and did not localize around the nucleus as their function would require. While the transport of melanosomes within melanocytes is mediated through actin- and myosin-based motor proteins, knowledge about their transport in keratinocytes is limited.[10] Experiments by McGrath *et al.* showed that keratinocytes from an affected person and those with Slac2-b knockdown exhibited disruption of keratin filament network and keratin filament aggregation.[2] Such features were also noted in our ultrastructural studies (Figure 3A,D) and thus underscore the role of Slac2-b in the maintenance of cytoskeletal integrity. Of note, exciting data have emerged during the last decade reporting new roles for intermediate filaments. In addition to their 'traditional' function in maintaining cellular scaffolding and integrity, keratin filaments help orchestrate the positioning and function of cellular organelles.[11] In skin, this role was demonstrated when melanosomes and mitochondria showed an accumulation and aberrant distribution in keratinocytes of patients with EBS-MP resulting from *KRT5* and *KRT14* mutations.[12] Also, haploinsufficiency of *KRT5* responsible for Dowling-Degos disease (DDD) affected melanosomes distribution in keratinocytes and led to reticulate hyperpigmentation.[13] Furthermore, autosomal dominant genodermatoses Naegelli-Franceschetti-Jadasson-syndrome (NFJS) and dermatopathia pigmentosa reticularis (DPR) are associated with pigmentary disturbances and mutations predicted to cause *KRT14* haploinsufficiency.[14] This study, in conjunction with reports regarding DDD, NFJS, DPR and EBS-MP, suggest that keratins play an important role in melanosomes uptake, transport and positioning in keratinocytes, a function thought to be limited to microtubules and microfilaments.[10] The mechanism how *EXPH5* mutations led to keratin filament network alteration is yet to be elucidated.

Conclusions

We report a female patient with a rare EBS-MP subtype resulting from a novel homozygous nonsense mutation in *EXPH5*. Based on this case, we propose the addition of the *EXPH5* gene into the EB consensus classification for the EBS-MP phenotype. The clinical presentation is characterized by mild generalized skin fragility, acral blistering improving with age, and most prominently late-onset MP features on her trunk and proximal extremities. Electron microscopy findings suggested a hypothesis underlying the epidermal pigmentary changes, implicating keratin filament impairment in the disturbance of pigment distribution.

Conflict of Interest Disclosures: none reported

Acknowledgements We would like to thank J. Zuiderveen and G. Meijer for their excellent technical assistance performing immunofluorescence staining and dr. G.F.H. Diercks for performing electron microscopy image analysis

Figures

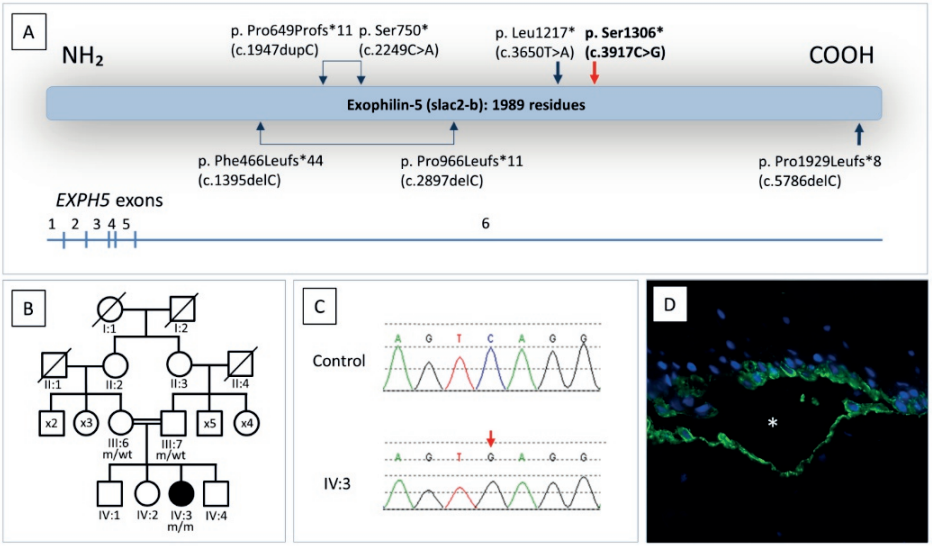

Figure 1. Mutation analysis and family pedigree of a patient with EBS-AR resulting from EXPH5 mutations

A, Schematic illustration of exophilin-5 protein and location of all EXPH5 mutations disclosed to date (dark blue) and mutation reported in current study (red). Homozygous mutations are depicted in thick arrows, whereas the compound heterozygous are shown in thin arrows (modified from McGrath et al., 2012). B, Clinical pedigree of the index patient. DNA analysis was performed in individuals lll:6, lll:7 and IV:3; m/wt, mutation/wildtype, or wt/wt, wildtype/wildtype, respectively, underlines the genotype of the particular individual. C, Sequencing of genomic DNA from individual IV:3 identified a homozygous mutation in EXPH5 in exon 6. A cytosine at position 3917 is substituted by a guanine (c.3917C>G). This substitution leads to a nonsense mutation (p. Ser1306). D, Immunofluorescence study with monoclonal antibody LL001 against keratin 14 identified a basal epidermal cleavage plane (asterisk) in lesional skin biopsy.*

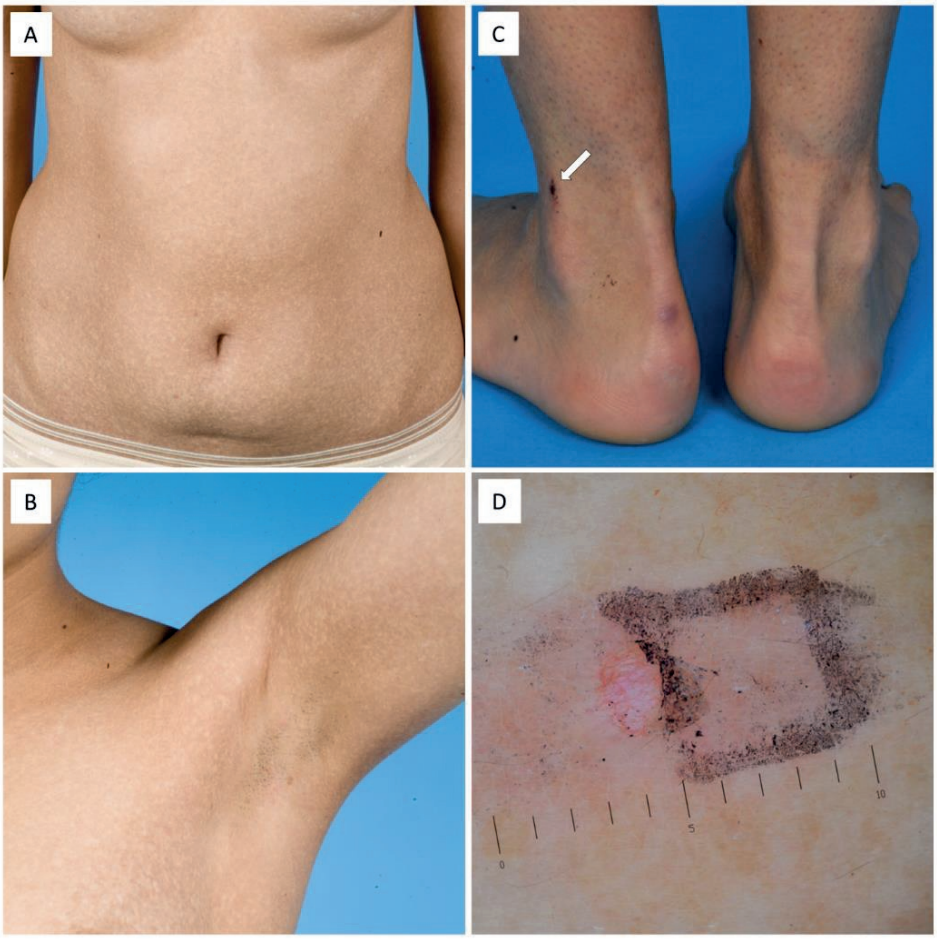

Figure 2. Clinical features

A, Mottled pigmentation on the trunk and B, axillary area. C, Trauma-induced hemorrhagic scale-crusts on the ankle. D, Dermatoscopy of positive ballpoint rub test reveals skin fragility upon application of mechanical stress.

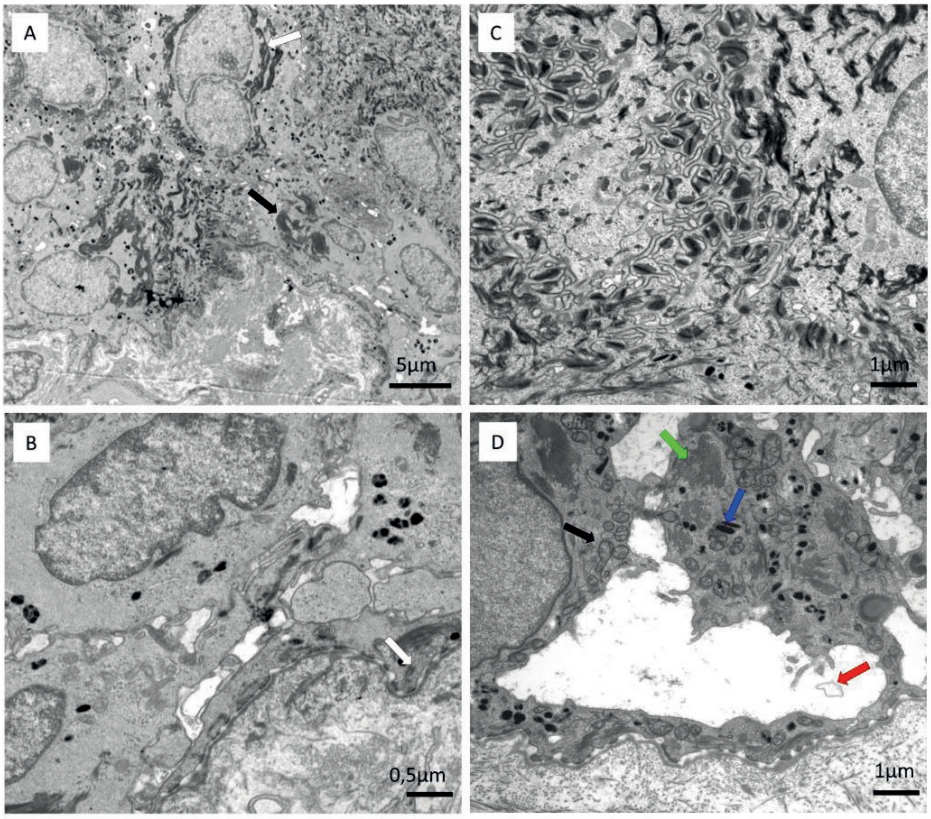

Figure 3. *Transmission electron microscopy studies*
A, Electron microscopy at low magnification shows basal keratinocytes that exhibit a "clear" aspect with an obvious deficiency in keratin filaments or aggregation of keratin filaments (black arrow). Some suprabasal keratinocytes show retraction of keratin filaments from the nuclear envelope creating a 'perinuclear halo' (white arrow). B, higher magnification depicts loss of desmosomes and cellular architecture of basal keratinocytes; note intracellular vesicles (white arrow). C, Striking pliability of the cell membrane containing an aggregation of desmosomes. D, Melanosomes (blue arrow) and mitochondria (black arrow) accumulate in a basal cell with clumping of keratin (green arrow); note the extracellular vesicle (red arrow).

References

1. Fine JD, Bruckner-Tuderman L, Eady RA, et al. Inherited epidermolysis bullosa: Updated recommendations on diagnosis and classification. *J Am Acad Dermatol*. 2014;70(6):1103-1126.

2. McGrath JA, Stone KL, Begum R, et al. Germline mutation in EXPH5 implicates the Rab27B effector protein Slac2-b in inherited skin fragility. *Am J Hum Genet*. 2012;91(6):1115-1121.

3. Liu L, Mellerio JE, Martinez AE, et al. Mutations in EXPH5 result in autosomal recessive inherited skin fragility. *Br J Dermatol*. 2014;170(1):196-199.

4. Pigors M, Schwieger-Briel A, Leppert J, et al. Molecular heterogeneity of epidermolysis bullosa simplex: Contribution of EXPH5 mutations. *J Invest Dermatol*. 2014;134(3):842-845.

5. Rashidghamat E, Ozoemena L, Liu L, McGrath JA, Martinez AE, Mellerio JE. Mutations in EXPH5 (exophilin-5) underlie a rare subtype of autosomal recessive epidermolysis bullosa simplex. *Br J Dermatol*. 2015.

6. Ostrowski M, Carmo NB, Krumeich S, et al. Rab27a and Rab27b control different steps of the exosome secretion pathway. *Nat Cell Biol*. 2010;12(1):19-30; sup pp 1-13.

7. Genome of the Netherlands Consortium. Whole-genome sequence variation, population structure and demographic history of the dutch population. *Nat Genet*. 2014;46(8):818-825.

8. Van Gele M, Dynoodt P, Lambert J. Griscelli syndrome: A model system to study vesicular trafficking. *Pigment Cell Melanoma Res*. 2009;22(3):268-282.

9. Echeverria-Garcia B, Vicente A, Hernandez A, et al. Epidermolysis bullosa simplex with mottled pigmentation: A family report and review. *Pediatr Dermatol*. 2013;30(6):e125-31.

10. Planko L, Bohse K, Hohfeld J, et al. Identification of a keratin-associated protein with a putative role in vesicle transport. *Eur J Cell Biol*. 2007;86(11-12):827-839.

11. Toivola DM, Tao GZ, Habtezion A, Liao J, Omary MB. Cellular integrity plus: Organelle-related and protein-targeting functions of intermediate filaments. *Trends Cell Biol*. 2005;15(11):608-617.

12. Uttam J, Hutton E, Coulombe PA, et al. The genetic basis of epidermolysis bullosa simplex with mottled pigmentation. *Proc Natl Acad Sci U S A*. 1996;93(17):9079-9084.

13. Betz RC, Planko L, Eigelshoven S, et al. Loss-of-function mutations in the keratin 5 gene lead to dowling-degos disease. *Am J Hum Genet*. 2006;78(3):510-519.